

This listing of claims will replace all prior versions, and listings, of claims in the application:

IN THE CLAIMS:

1. (Currently amended) A method for preparing a nucleic acid binding protein that binds to a target nucleotide sequence, wherein the binding protein comprises a plurality of zinc fingers of the Cys2-His2 class, wherein the method comprises:
- i) selecting a quadruplet within the target nucleotide sequence;
  - ii) designing the binding protein such that binding of a zinc finger to the quadruplet is obtained by choosing the sequence of particular residues of the zinc finger depending on the nucleotide sequence of the quadruplet, as follows:
    - a) if base 4 in the quadruplet is A, then position +6 in the  $\alpha$ -helix is Glu, Asn or Val;
    - b) if base 4 in the quadruplet is C, then position +6 in the  $\alpha$ -helix is Ser, Thr, Val, Ala, Glu or Asn
    - c) if base 4 in the quadruplet is G, then position +6 in the  $\alpha$ -helix is Arg or Lys;
    - d) if base 4 in the quadruplet is T, then position +6 in the  $\alpha$ -helix is Ser, Thr, Val or Lys;
  - iii) synthesizing a polynucleotide encoding the binding protein of (ii);
  - iv) introducing the polynucleotide of (iii) into a cell; and
  - v) incubating the cell under conditions in which the encoded nucleic acid binding protein is expressed.

2. (Currently Amended) A method according to claim 1, wherein ~~binding to base 4 is G or T of the quadruplet by a zinc finger is additionally determined as follows:~~

- ~~\_\_\_\_\_ c) if base 4 in the quadruplet is G, then position +6 in the  $\alpha$ -helix is Arg or Lys;~~  
~~\_\_\_\_\_ d) if base 4 in the quadruplet is T, then position +6 in the  $\alpha$ -helix is Ser, Thr, Val or Lys.~~

3. (Currently amended) A method for preparing a nucleic acid binding protein that binds to a target nucleotide sequence, wherein the binding protein comprises a plurality of zinc fingers of the Cys2-His2 class, wherein the method comprises:

- i) selecting a quadruplet within the target nucleotide sequence;  
ii) designing the binding protein such that binding of a zinc finger to the quadruplet is obtained by Choo et al. using the sequence of particular residues of the zinc finger depending on the nucleotide sequence of the quadruplet, as follows:

- a) if base 4 in the quadruplet is G, then position +6 in the  $\alpha$ -helix is Arg or Lys;  
b) if base 4 in the quadruplet is A, then position +6 in the  $\alpha$ -helix is Glu, Asn or Val;  
c) if base 4 in the quadruplet is T, then position +6 in the  $\alpha$ -helix is Ser, Thr, Val or Lys;  
d) if base 4 in the quadruplet is C, then position +6 in the  $\alpha$ -helix is Ser, Thr, Val, Ala, Glu or Asn;

e) if base 3 in the quadruplet is G, then position +3 in the  $\alpha$ -helix is His;

f) if base 3 in the quadruplet is A, then position +3 in the  $\alpha$ -helix is Asn;

g) if base 3 in the quadruplet is T, then position +3 in the  $\alpha$ -helix is Ala, Ser or Val; ~~provided that if it is Ala, then one of the residues at -1 or +6 is a small residue;~~

h) if base 3 in the quadruplet is C, then position +3 in the  $\alpha$ -helix is Ser, Asp, Glu, Leu, Thr or Val;

i) if base 2 in the quadruplet is G, then position -1 in the  $\alpha$ -helix is Arg;

j) if base 2 in the quadruplet is A, then position -1 in the  $\alpha$ -helix is Gln;

k) if base 2 in the quadruplet is T, then position -1 in the  $\alpha$ -helix is His or Thr;

l) if base 2 in the quadruplet is C, then position -1 in the  $\alpha$ -helix is Asp or His;

m) if base 1 in the quadruplet is G, then position +2 is Glu;

n) if base 1 in the quadruplet is A, then position +2 Arg or Gln;

o) if base 1 in the quadruplet is C, then position +2 is Asn, ~~Gln, Arg,~~  
Gln, Arg, His or Lys;

(p) if base 1 in the quadruplet is T, then position +2 is Ser or Thr

iii) synthesizing a polynucleotide encoding the binding protein of (ii);

iv) introducing the polynucleotide of (iii) into a cell; and

v) incubating the cell under conditions in which the encoded nucleic acid binding protein is expressed.

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4. (Currently amended) A method according to ~~any preceding~~ claim 3, wherein the each zinc finger has the general primary structure  
 $X^a \text{Cys } X_{2-4} \text{Cys-} X_{2-3} \text{-Phe-} X^c \text{-X-X-X-X-Leu-X-X-His-X-X-} X^b \text{His-linker}$  (SEQ ID NO: 3)

-1 1 2 3 4 5 6 7 8 9

wherein X (including  $X^a$ ,  $X^b$  and  $X^c$ ) is any amino acid.

5. (Previously amended) A method according to claim 4 wherein  $X^a$  is Phe/Tyr-X or Pro-Phe/Tyr-X.

6. (Previously amended) A method according to claim 5 wherein  $X_{2-4}$  is selected from any one of:  
Ser-X, Glu-X, Lys-X, Thr-X, Pro-X and Arg-X.

7. (Previously amended) A method according to claim 4 wherein  $X^b$  is Thr or Ile.

8. (Previously amended) A method according to claim 4 wherein  $X_{2-4}$  is Gly-Lys-Ala, Gly-Lys-Cys, Gly-Lys-Ser, Gly-Lys-Gly, Met-Arg-Asn or Met-Arg.

9. (Previously amended) A method according to claim 4 wherein the linker is Thr-Gly-Glu-Lys (SEQ ID NO: 4) or Thr-Gly-Glu-Lys-Pro (SEQ ID NO: 5).

10. (Previously amended) A method according to claim 4 wherein position +9 is Arg or Lys.

11. (Previously amended) A method according to claim 4 wherein positions +1, +5 and +8 are not occupied by any one of the hydrophobic amino acids, Phe, Trp or Tyr.

12. (Previously amended) A method according to claim 11 wherein positions +1, +5 and +8 are occupied by the residues Lys, Thr and Gln respectively.

13. (Previously amended) A method for preparing a nucleic acid binding protein of the Cys2-His2 zinc finger class which binds a target nucleic acid sequence, comprising the steps of:

- a) selecting a model zinc finger domain from the group consisting of naturally occurring zinc fingers and consensus zinc fingers; and
- b) mutating the finger according to the rules set in any one of claims 1 to 3.

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14. (Currently amended) A method according to claim 13, wherein the model zinc finger is a consensus zinc finger whose structure is selected from the group consisting of the consensus structure Pro-Tyr-Lys-Cys-Pro-Glu-Cys-Gly-Lys-Ser-Phe-Ser-Gln-Lys-Ser-Asp-Leu-Val-Lys-His-Gln-Arg-Thr-His-Thr-Gly (SEQ ID NO: 6), and the consensus structure Pro-Tyr-Lys-Cys-Ser-Glu-Cys-Gly-Lys-Ala-Phe-Ser-Gln-Lys-Ser-Asn-Leu-Thr-Arg-His-Gln-Arg-Ile-His-Thr-Gly-Glu-Lys-Pro (SEQ ID NO: 7).

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15. (Currently amended) A method according to claim 13 wherein the model zinc finger is a naturally-occurring zinc finger whose structure is selected from one finger of a protein selected from the group consisting of Zif 268 [~~Eldred-Erickson et al., (1996) Structure 4:1171-1180~~], GLI, Tramtrack and YY1.

16. (Original) A method according to claim 15 wherein the model zinc finger is finger 2 of Zif 268.

17. (Previously amended) A method according to claim 3 wherein the binding protein comprises two or more zinc finger binding motifs, placed N-terminus to C-terminus.

18. (Previously amended) A method according to claim 14, wherein the N-terminal zinc finger is preceded by a leader peptide having the sequence Met-Ala-Glu-Glu-Lys-Pro (SEQ ID NO: 8).

19. (Previously amended) A method according to claim 13 wherein the nucleic acid binding protein is obtained by recombinant nucleic acid technology, the method comprising the steps of:

a) preparing a nucleic acid coding sequence encoding two or more model zinc finger domains, placed N-terminus to C-terminus;

b) inserting the nucleic acid sequence into a suitable expression vector;  
and

c) expressing the nucleic acid sequence in a host organism in order to obtain the nucleic acid binding protein.

20. (Previously amended) A method according to claim 3 comprising the additional steps of subjecting the nucleic acid binding protein to one or more rounds of randomisation and selection in order to improve the characteristics thereof.

21. (Original) A method according to claim 20, wherein the randomisation and selection is carried out by phage display technology.

22. (Previously amended) A method according to claim 21, comprising the steps of:

- a) preparing a nucleic acid construct which express a fusion protein comprising the nucleic acid binding protein and a minor coat protein of a filamentous bacteriophage;
- b) preparing further nucleic acid constructs which express a fusion protein comprising a selectively mutated nucleic acid binding protein and a minor coat protein of a filamentous bacteriophage;
- c) causing the fusion proteins defined in steps (a) and (b) to be expressed on the surface of bacteriophage transformed with the nucleic acid constructs;
- d) assaying the ability of the bacteriophage to bind the target nucleic acid sequence and selecting the bacteriophage demonstrating superior binding characteristics.

23. (Previously amended) A method according to claim 20 wherein the nucleic acid binding protein is selectively randomised at any one of positions +1, +5, +8, -1, +2, +3 or +6.

24. (Original) A method according to claim 23, wherein, in the nucleic acid binding protein, position +6 of a zinc finger and positions -1, +1, +2 and +3 of an adjacent zinc finger are randomised.

25. (Previously amended) A method for determining the presence of a target nucleic acid molecule, comprising the steps of:

- a) preparing a nucleic acid binding protein by the method of claim 3 which is specific for the target nucleic acid molecule;
- b) exposing a test system comprising the target nucleic acid molecule to the nucleic acid binding protein under conditions which promote binding, and removing any nucleic acid binding protein which remains unbound;
- c) detecting the presence of the nucleic acid binding protein in the test system.

26. (Original) A method according to claim 25, wherein the presence of the nucleic acid binding protein in the test system is detected by means of an antibody.

27. (Previously amended) A method according to claim 25 wherein the nucleic acid binding protein, in use, is displayed on the surface of a filamentous bacteriophage and the presence of the nucleic acid binding protein is detected by detecting the bacteriophage or a component thereof.

28. (Previously amended) A synthetic nucleic acid binding protein whose design incorporates a method according to claim 3.



29. (Original) A nucleic acid encoding a nucleic acid binding protein according to claim 28.

30. (Original) A host cell transformed with a nucleic acid according to claim 29.

31. Canceled.

32. (Previously added) The method of claim 3, wherein a plurality of overlapping quadruplets are selected within the target sequence.